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THE UNIVERSITY OF ALBERTA

THE EFFECT OF THERAPEUTIC ULTRASOUND ON THE LEVEL OF BLOOD SUGAR IN THE HUMAN BODY

BY

DAVID J. MAGEE (C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Effect of Therapeutic Ultrasound on the Level of Blood Sugar in the Human Body" submitted by David J. Magee in partial fulfilment of the requirements for the degree of Master of Science.

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Date SEPTEMBER 26, 1975

DEDICATION

I would like to dedicate this manuscipt to my wife, Bernice, who through her patience and assistance made it possible to continue the project through to completion.

ABSTRACT

The purpose of this study was to determine what effect, if any, the application of therapeutic ultrasound had on the level of blood sugar in the human body using an ultrasound machine with a frequency of eight hundred and seventy kilocycles.

Two doses of ultrasound were used, one thermal and one non-thermal. There were four test groups - a control, a placebo, and one receiving a thermal dose of ultrasound, and one receiving a subthermal dose of ultrasound. Each subject in each group had four blood samples taken - one immediately prior to application of ultrasound, one immediately after application of ultrasound, one one half hour after application of ultrasound, and one one hour after application of ultrasound. The subjects were selected by means of a random table and a two-way analysis of variance with repeated measures on one factor was used to analyze the data. The results indicated that the application of 0.5 watts and 1.5 watts of continuous therapeutic ultrasound for ten minutes do not alter the level of blood sugar in the human body significantly.

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CHAPTER I

Statement of the Problem

Introduction

High frequency sound waves (ultrasound) have been used for many years in detection devices, diagnostic instruments and as a therapeutic modality. Initially, ultrasound was employed in World War I in detection instruments to locate submarines. Between the world wars, its use in the treatment of disease or injuries slowly advanced and, after World War II, the medical aspects of ultrasound moved to the foreground (1,2).

The therapeutic ultrasound apparatus makes use of an oscillating generator which causes a quartz crystal in the treatment head to vibrate at a high frequency. Therapeutic ultrasound machines usually have a frequency of 1000 kilohertz or 870 kilohertz.

The production of ultrasonic waves depends on an electrical current and a circular quartz crystal disc. The application of a selective alternating current to the crystal causes the crystal to expand and contract in time with the cycles of electric current. This action is termed the Reverse Piezoelectric Effect. Each crystal vibrates at its own "natural" frequency and so a generator in the ultrasound apparatus must be tuned to the crystal (3,4). The amplitude

of the crystal deformation is extremely small (one to two microns), but it is sufficient to translate the electrical oscillations of the alternating current into mechanical vibrations. These mechanical vibrations set up sound waves which pass through the protective metal cap of the treatment head into the air and surrounding tissues (3). Ultrasonic waves cannot be transmitted in a vacuum and in order to get maximum absorption into the tissues, a coupling agent is necessary. There are many coupling agents available, some are more efficient than others in the transmission of sound waves (5).

Sound energy exhibits some of the properties of light energy; it may be reflected refracted and absorbed. Reflection occurs at boundaries or interfaces between tissues of different densities while refraction occurs when the angle of incidence is fifteen degrees or greater to the interface of tissues. Absorption varies both with the frequency and the nature of the tissue. The beam of ultrasound waves does not terminate during transmission but goes on with ever decreasing power (1,6).

The effects produced by the ultrasound are mainly due to the mechanical action of the sound waves on the tissues. The vibrating waves of ultrasound cause an agitation or shaking of the tissues at a microscopic level. The production of heat as a result of the agitation of adjacent body particles leads to a local increase in blood flow through the area resulting in increased permeability of the tissues. To

accomplish this increase in blood flow, the sound energy must be applied continuously and have an intensity of at least one watt per square centimetre (1,7). Intensities below this are considered non thermal and those above this intensity are considered thermal. Pulsed ultrasound allows for the dissipation of heat between two immediately consecutive pulses (1). The heating effect of the ultrasound enhances the passage of ions through cell membranes. The movement of the ions is further enhanced by the stirring effect of the high frequency sound waves and the acoustic or fluid streaming. The streaming is the result of compression and decompression caused by the sound waves. The forces producing the density and rarefraction are unequal and this resultant imbalance aids the unidirectional movement of the particles (1,7,9,10,11,12,22).

Mechanically, the ultrasound energy causes collagen and fibrous tissue to be pulled apart and pushed together resulting in tissue which is softer and more pliable (1). Application of ultrasound results in an increase in adenosine triphosphate activity and a greater increase in its interaction with calcium than would be evident under normal circumstances when the area was not treated with ultrasound (8).

Although ultrasound has many beneficial effects in the treatment of medical conditions by its actions on the tissues, it also may have a few harmful side effects if not applied with care. An excessive amount of heat may be produced particularly at tissue interfaces, resulting in a burn to the patient

which may be of the third degree variety (12,13,23). Taylor and Pond (24) found that damage to the liver upon the application of ultrasound was frequency dependent. They felt that the inverse relationship between damage and frequency was not thermal related.

Nervous tissue, because it absorbs more ultrasound than any other tissues, may be more susceptible to damage especially at high doses of ultrasound energy (8). For this reason ultrasound must be given with care in the areas of the eyes, brain, spinal cord and nerve trunks. Several authors (1,9,14,15,16) state that ultrasonic energy has a specific effect on the autonomic nervous system especially the sympathetic system which is stimulated.

Ultrasound may also have an effect on the chemistry of the blood influencing blood sugar levels and increasing total oxygen uptake (1,9,17,18).

Purpose of the Study:

Summer and Patrick (1) and El'piner (18) state that ultrasound has an effect on blood sugar levels in a person but they show no data or references to collaborate this statement. Summer and Patrick state:

> In a number of cases the drop in blood sugar was as as much as from 101 to 82 mg%.
> The blood sugar level in 40 to 60% of patients insonated decreases immediately after ultrasonic treatment and may show a decrease which is 15.7 mg% (average), but a maximum of 57 mg% is on record.

They do not state the dosage or frequency of ultrasound used, or the time period of application. These statements

could have serious implications in the application of therapeutic ultrasound.

Thus, the purpose of this study is to determine what effect, if any, the application of therapeutic ultrasound has on the level of blood sugar in the human body.

Deliminations:

1. This study is limited to two intensities of ultrasound, one thermal (1.5 watts per square centimetre), and one subthermal (0.5 watts per square centimetre), both of which are within the normal clinical range of 0.1 to 3.0 watts per square centimetre.

2. The time limit of exposure for each subject is ten minutes. This time was selected to give a sufficient dosage and is also considered by many to be the maximum clinical treatment time of ultrasound.

3. The area of application of ultrasound is limited to the right forearm of each subject and the area receiving ultrasound is four times the size of the soundhead. This area was chosen because it is easily accessible and is an area which is treated with ultrasound in musculoskeletal conditions. The size of the area irradiated was selected as it is the usual area treated in the application of therapeutic ultrasound.

4. The study is limited to forty healthy subjects (twenty female, and twenty male).

5. An ultrasound machine with a frequency of 870

kilocycles per second is used as this frequency is the one most frequently used clinically.

6. The selection of time intervals for the taking of blood samples is set at immediately before treatment, immediately after treatment, thirty minutes after treatment, and sixty minutes after treatment.

7. For the blood analysis method used, the normal fasting blood sugar levels range from sixty-five to 105 milligrams per 100 millilitres of blood (19,20).

Definition of Terms:

1. Average Intensity (in watts per square centimetre): the total output in watts divided by the effective radiated area in centimetres.

2. Fasting Blood Sugar Level: the level of blood sugar when there is an equilibrium between the rate at which glucose forms in the liver, and the rate at which glucose is used in the tissue.

3. Thermal Dosage of Ultrasound: A dosage of ultrasound sufficient to cause a subjective feeling of heat when applied using a standard clinical technique.

4. Non thermal Dosage of Ultrasound: A dosage of ultrasound not sufficient to cause a subjective feeling of heat when applied using a standard clinical technique.

Basic Assumptions:

This study will proceed on the following basic assumptions:

1. The actual intensity output of the ultrasound machine and the reading on the intensity meter of the ultrasound machine are within ± fifteen per cent of each other (21).

2. The area of application of ultrasound is sufficient to cause irradiation of the blood with ultrasonic waves.

3. Under the conditions which the study will be conducted, the changes which will be noted are the result of ultrasonic energy applied to the tissues.

Hypothesis

1. Verbal Hypothesis: If continuous ultrasound energy with a frequency of 870 kilocycles per second is applied at the intensities of 0.5 and 1.5 watts per square centimetre with continuous output to the right forearm of normal healthy subjects for a period of ten minutes using aquasonic gel ® as a coupling agent, there will be no significant change or effect on the level of blood sugar in the blood at the 0.5 level of significance immediately after, thirty minutes after or one hour after treatment.

2. Symbolic Null Hypothesis:

$$\mu_1 = \mu_2 = \mu_3 = \mu_4$$

KEY:

μ_1 : Level of blood sugar before treatment

μ_2 : Level of blood sugar immediately after treatment

μ_3 : Level of blood sugar 30 minutes after treatment

μ_4 : Level of blood sugar 60 minutes after treatment

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CHAPTER II

Review of Literature

During the last three decades there has been a vast amount of literature pertaining to ultrasonics. The review of literature attempts to deal first with the normal metabolism of glucose; secondly with factors affecting the level of blood sugar, and finally the effect of ultrasound on the blood.

Normal Metabolism of Glucose

There are several processes involved in the metabolism of glucose, some of them anaerobic, and some aerobic. The primary anaerobic process is called Glycolysis or the Embden-Meyerhoff Pathway (1,2,3,4,5). It consists of four stages. In the first stage, glucose is converted to fructose 1,6-diphosphate in three steps by the action of three successive enzymes. Glucose is acted upon by the enzyme glucokinase resulting in glucose 6-phosphate, which in turn is acted upon by glucose phosphate isomerase resulting in fructose 6-phosphate. Finally, the fructose 6-phosphate, which involves the release of energy as does step one, is converted to fructose 1,6-diphosphate by the enzyme phosphofructokinase.

The second stage of Glycolysis involves the splitting of fructose 1,6-diphosphate by the enzyme aldolase to produce

dehydroxyacetone phosphate and glyceraldehyde 3-phosphate (1,2,5).

The glyceraldehyde 3-phosphate is used in the third stage of Glycolysis. This is the primary energy yielding stage and consists of three phases. First, the glyceraldehyde 3-phosphate is converted to 3-phosphoglyceric acid. This portion of the process does not require an enzyme, while the second phase requires the enzyme glyceraldehyde 3-phosphate dehydrogenase, and the third phase involves the enzyme phosphoglycerate kinase (1,2,5).

The final stage of Glycolysis involves the recovery of phosphate groups with the final product formed being pyruvic acid or lactic acid. If the process is anaerobic, lactic acid is produced; if the process is aerobic, or involves an aerobic part, pyruvic acid is produced. This final stage of Glycolysis also involves three phases with each phase having it own enzyme-phosphoglyceromutase, phosphopyruvate hydratase, and pyruvate kinase respectively (1,2,5,6).

The other anaerobic process is the Cori Cycle which is actually a part of Glycolysis. This process occurs when energy is required for vigorous exercise of brief duration and the supply of oxygen is insufficient to meet the demands of the tissues. Glycogen in the muscle is broken down by Glycolysis into lactic acid which enters the blood. The liver then converts the lactic acid to liver glycogen by a process called gluconeogenesis involving the conversion of fat and protein to carbohydrate. The liver glycogen is then

STAGE 1: (a) Glucose + ATP $\xrightarrow{\text{Glukokinase}}$ Glucose 6-Phosphate + ADP

(b) Glucose 6-Phosphate $\xrightarrow{\text{Glucose-phosphate Isomerase}}$ Fructose 6-Phosphate

(c) Fructose 6-Phosphate + ATP $\xrightarrow{\text{Phosphofructokinase}}$ Fructose 1,6-Diphosphate + ADP

STAGE 2: Fructose 1,6-Disphosphate $\xrightarrow{\text{Aldolase}}$ Dihydroxy Acetone Phosphate + Glyceraldehyde 3-Phosphate

STAGE 3: (a) Glyceraldehyde 3-Phosphate + NAD + Inorganic Phosphate $\xrightarrow{\text{Glyceraldehyde 3-Phosphate Dehydrogenase}}$ 1,3-Diphosphoglyceric Acid + NADH

(b) 1,3-Diphosphoglyceric Acid + ADP $\xrightarrow{\text{Phosphoglycerate kinase}}$ 3-Phosphoglyceric Acid + ATP

STAGE 4: (a) 3-Phosphoglyceric Acid $\xrightarrow{\text{Phosphoglyceromutase}}$ 2-Phosphoglyceric Acid

(b) 2-Phosphoglyceric Acid $\xrightarrow{\text{Enolase}}$ Phosphoenolpyruvic Acid

(c) Phosphoenolpyruvic Acid + ADP $\xrightarrow{\text{Pyruvate Kinase}}$ Pyruvic Acid + ATP

FIGURE 1: FLOW CHART FOR GLYCOLYSIS (AEROBIC STATE)

split up into free glucose by a process called glycogenolysis resulting in free glucose in the blood. This free glucose is used to replenish muscle glycogen (1,5).

The primary aerobic process involved in the metabolism of glucose is the Citric Acid Cycle or Krebs Cycle (1,2,3,4,5). As with Glycolysis, the Krebs Cycle is divided into four stages. In the primary stage, acetyl-coenzyme A combines with oxaloacetic acid through the action of the enzyme citrate synthase resulting in citric acid. The citric acid is then acted upon by the enzyme aconitate hydratase to produce isocitric acid.

The second stage involves isocitric acid being converted to succinic acid. The succinic acid loses a carbon dioxide molecule by decarboxylation resulting in α-oxoglutaric acid. These two steps of the second stage are catalyzed by the enzyme isocitrate dehydrogenase. The α-oxoglutaric acid undergoes oxidative decarboxylation resulting in succinyl coenzyme-A. This change is due to the enzyme α-oxoglutarate dehydrogenase (1,2,4,5).

The third stage of Krebs Cycle involves the splitting of succinyl coenzyme-A into succinic acid and free coenzyme-A. No enzymes are involved in this stage.

The final stage, like that of Glycolysis, involves three phases. The succinic acid is broken down by the enzyme succinate dehydrogenase to produce fumaric acid. The fumaric acid is acted on by fumarate hydratase resulting in malic acid. Finally, malic acid is dehydrogenated by the enzyme

STAGE 1: (a) Pyruvic Acid + Coenzyme A ⟶ Acetyl Coenzyme A + ATP + CO_2

(b) Acetyl Coenzyme A + Oxaloacetic Acid $\xrightarrow{\text{Citrate Synthase}}$ Citric Acid + Coenzyme A

(c) Citric Acid $\xrightarrow{\text{Aconitate Hydratase}}$ Isocitric Acid

STAGE 2: (a) Isocitric Acid + NAD + ATP $\xrightarrow{\text{Isocitrate Dehydrogenase}}$ α-oxoglutane Acid + NADH

(b) α-oxoglutaric Acid + Coenzyme A $\xrightarrow{\text{α-oxoglutarate Dehydrogenase}}$ Succinyl Coenzyme A

STAGE 3: Succinyl Coenzyme A + ADP + Inorganic Phosphate ⟶ Succinic Acid + ATP

STAGE 4: (a) Succinic Acid $\xrightarrow{\text{Succinate Dehydrogenase}}$ Fumaric Acid

(b) Fumaric Acid $\xrightarrow{\text{Fumarate Hydratase}}$ Malic Acid

(c) Malic Acid + NAD $\xrightarrow{\text{Malate Dehydrogenase}}$ Oxaloacetic Acid + NADH

FIGURE 2: FLOW CHART FOR KREBS CYCLE

malate dehydrogenase to give oxaloacetic acid, resulting in a completed Krebs cycle (1,2,4).

The other aerobic process is the Pentose Phosphate Shunt or the Hexose Phosphate Shunt which provides an alternate pathway for glucose breakdown (1,2,4). This cycle process involves two phases. The first phase involves the oxidation of glucose 6-phosphate into 6-phosphogluconic acid by the action of the enzyme glucose-6-phosphate dehydrogenase. The 6-phosphogluconic acid loses a carbon dioxide molecule resulting in a pentose called ribulose 5-phosphate. The second phase of the cycle, or conversion of pentose to hexose involves three main types of reaction. The first, transkitolation, involves a reaction between two pentose phosphate molecules. The second, transaldolation, involves the reaction between a triose phosphate and a heptose phosphate molecule resulting in a hexose phosphate and a tetrose phosphate. The third type of reaction, the aldolase reaction, combines two identical molecules to give one molecule (example: two triose phosphate molecules combine to give one hexose diphosphate molecule) (1,2,4).

Factors Affecting the Level of Blood Sugar

There are several factors which may lower the level of blood sugar in the human body. These include satiety, glucose diffusion in the extracellular fluid, muscular exercise, and insulin (4,7).

Insulin, a hormone, comes from the β-cell of the islets

(a) Glucose 6-Phosphate $\xrightarrow{\text{Glucose 6-Phosphate Dehydrogenase}}$ 6 Phosphogluconic Acid

(b) 6 Phosphogluconic Acid $\xrightarrow{\text{6-Phosphogluconate Dehydrogenase}}$ Ribose 5-Phosphate

(c) Ribose 5-Phosphate is then converted to Hexose by 3 Main Reactions:

1. Transketolation - Example: $C_5 + C_5 \rightleftharpoons C_3 + C_7$
2. Transaldolation - Example: $C_3 + C_7 \rightleftharpoons C_6 + C_4$
3. Aldolase Reaction - Example: $C_3 + C_3 \rightleftharpoons C_6$

FIGURE 3: PENTOSE PHOSPHATE SHUNT

of Langerhans in the pancreas. Its function is to facilitate the entry of glucose into muscle cells and adipose tissue and it promotes the phosphorylation of glucose in the liver. The phosphorylation is accomplished by insulin's action on the glucose transportation system (1,3,4,9,10,11,12). Insulin helps to keep the blood sugar level from going too high and an excessive amount can lead to hypoglycemia (1,4). The release of insulin is stimulated by the presence of sugars in the body, amino acids and ketones, the hormones glucagon and growth hormone, and cyclic AMP. Inhibition of insulin release is due to starvation, the hormones adrenaline and noradrenaline, and 2-Deoxyglucose (3).

Factors which tend to raise the level of blood sugar in the human body include hunger, glucose absorption from the gut, hepatic glucogenolysis, the hormones adrenaline, glucagon, growth hormone, glucocorticoids and gluconeogensis in the liver (4).

The liver attempts to maintain a normal level of blood sugar. It does this by regulating new glucose formation and by removing glucose from the blood. However, if the splitting up of glucose occurs in the liver (glucogenolysis), then there will be a raise in blood sugar (4).

Adrenaline, a hormone from the adrenal medulla, stimulates glycogenolysis in the liver and is used by the body primarily in an emergency when violent muscle action is required. It stimulates the release of glucose from storage by acting on muscle stores as well as liver stores,

and stimulates the formation of a specific cyclic AMP "the second messenger" (1,2,4,5,6).

Glucagon is the first line of defence against hypoglycemia just as insulin is the first line of defence against hyperglycemia. Glucagon comes from the α-cells of the islets of Langerhans in the pancreas and stimulates insulin release (1,6,8). Like adrenaline, it stimulates glycogenolysis in the liver and the formation of cyclic AMP which increases the breakdown of glycogen into glucose (1,2,5,6). As well, it stimulates gluconeogenesis in the liver (2,4). Gluconeogenesis is the formation of sugar from non-carbohydrate molecules such as protein.

Growth hormone, which comes from the anterior pituitary, is an insulin antagonist. It causes a lack of response to insulin so that glucose is spared when it is in short supply. As well, it favours protein anabolism (1,2,6,9).

Glucocorticoids, chiefly cortisol, which come from the adrenal cortex, are insulin antagonists and inhibit utilization of glucose by suppressing phosphorylation (1,6,7). This facilitates the actions of glucagon, adrenaline and growth hormone. The cortisol acts as a slow second line of defence against hypoglycemia (1).

Extracts of the anterior pituitary, such as ACTH, can result in hyperglycemia as they promote cortisol secretion (2,4). Thyroxine, a thyroid hormone, increases the rate of glucose absorption from the gut and stimulates glycogenolysis (1,2,4,5).

The Central and Autonomic Nervous Systems also play a role in maintaining the level of blood sugar. Before the Central Nervous System is stimulated to action, however, there must be a drop in blood sugar. The drop in blood sugar results in an increase in sympathetic activity and the rate of adrenaline secretion increases. The hormone and sympathetic impulses act together to stimulate glycogenolysis (4,14,15,16).

Effect of Ultrasound on the Blood

Several studies have been done on ultrasound and its effect on blood but most of these have dealt with blood flow. Most of these studies (14,17,18,19,20,21,22,23) found that blood flow was not affected until the muscle temperature increased. This increased flow remained for at least sixty minutes according to one study (17). It was found that in order to get a consistent increase in blood flow, the intensity of the ultrasound would have to be 3.0 to 3.5 watts per square centimetre, and the treatment time fifteen minutes (14,20). Imig (18) reported that subthermal doses of ultrasound had no effect on blood flow. Lota (17) found that with the local application of ultrasound (intensity: 1.0 watts per square centimetre for five minutes), maximum blood flow did not occur until after the treatment was completed and the flow was still slightly elevated after sixty minutes. Other authors (31,36) support this finding.

The absorption of ultrasound energy in normal blood is

proportional to the frequency of the beam - the higher the frequency, the greater the absorption (14). It is the presence of protein molecules which accounts for this absorption (24,25,26,27,28). Enzymes, which are primarily protein, may also be affected by an ultrasonic field. El'piner (25) felt that the effect of ultrasound on enzymes depended on the properties of gas with which the investigated solution was saturated. For example, enzymes were inactivated by ultrasound when oxygen was present. As well, insulin could be broken down by the splitting off of small peptides or individual amino acids on exposure to ultrasound (25). On the other hand, ultrasound may enhance the action of enzymes, particularly with cell membranes (25,29).

Ultrasonic energy improves gas exchange across membranes and increase cellular transmembrane permeability (14,19,25, 29). It does this by causing a local increase in temperature, its stirring action, and its acoustic streaming effect (15, 26,30,31,32,33,34).

According to Summer and Patrick (14) the blood sugar level in forty to sixty per cent of the patients treated with ultrasound decreases immediately after the treatment and may show a decrease of 15.7 milligram per cent (average) in the blood sugar level. These authors feel this may be due, in part, to the action of ultrasonic waves on the Autonomic Nervous System. Bickford and Duff (20) felt any changes were due to the ultrasonic waves and not due to the massaging effect of the treatment head which might possibly stimulate

the Autonomic Nervous System. El'piner (25) felt that the ultrasonic field resulted in chemical changes in carbohydrates if the dosage of ultrasound was five to seven watts per square centimetre and the treatment time ten to fifteen minutes, both of which exceed therapeutic treatment parameters. The changes occurring in the carbohydrates were partly due to heat and partly due to the mechanical vibration of the ultrasonic energy. Ziminey and Head (35) found that glycogen was reduced in all tissues irradiated by ultrasound.

In summary, it appears evident that because of the action of ultrasound on substances such as hormones and carbohydrates, and areas of the body such as the cell membrane and nervous systems, there are many places in the chain of glucose metabolism where ultrasound could potentially act should the reported alteration in blood sugar in the human body be substantiated and an explanation of possible mechanisms need to be offered.

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CHAPTER III

Methods and Procedures

Apparatus

The ultrasound unit was a Burdick model UT-420A which operated at a frequency of 870 kilohertz utilizing a ceramic transducer. A plastic template was used to mark out the area of ultrasound application and aquasonic gel ^{R} was used as a coupling agent (1). The timing of the ultrasound treatment was by means of a Gra-lab Timer (model 171).

Blood samples were taken using 21 guage sterile Venoject blood collecting needles which were 1 1/2 inches long, B-D vacutainers with grey stopper (five milligrams of thymol and fifty milligrams of sodium floride), and vacutainer holders. The blood samples were analyzed at the University of Alberta Hospital Laboratory utilizing a Technicon AutoAnalyzer system.

Sample Selection

The sample consisted of forty healthy volunteer subjects (twenty male and twenty female). The subjects were divided into four groups (control, placebo, subthermal, and thermal). Each group consisted of ten subjects (five male and five female) selected by means of a random table. Once selected,

the subjects were instructed to refrain from eating, smoking, or drinking for at least eight hours prior to the testing. In this way, the blood sugar would have had time to stabilize to a resting state as the blood glucose level can go as high as 140 to 180 milligrams per 100 millilitres of blood immediately following a meal (2). As well, it has been found that in fasting subjects, the arterial blood glucose is only two or three milligrams per one hundred millilitres higher in arterial blood than in venous blood, because during fasting, the tissues take very little sugar from the blood so that blood sugar levels in all of the blood of the body is as near to equal as possible (3). Prior to being tested, all of the subjects were given a medical questionnaire (see appendix A) to help rule out any possibility of circulatory problems such as thrombosis, diabetes, varicose veins and heart disease. No subjects were rejected from the test because of their medical history. Two subjects were rejected for not fasting for eight hours.

Testing Procedure

On arrival at the testing lab, subjects had the testing procedure fully explained to them and then were asked to sign an Informed Consent Form (see appendix A). Each subject was instructed to lie on the padded plinth for one-half hour prior to the test being performed (4). The subject lay with both arms exposed and hands in the supinated position. While the subject rested the area of the right arm to receive

the application of ultrasound was cleaned with alcohol to remove all traces on natural grease. A plastic template was used to measure out the area of ultrasound application so that this would be uniform for all subjects. The template size was four times the size of the ultrasound treatment head.

During the rest period, each subject was tested for hot-cold sensation, using two test tubes, one containing hot water, and one containing cold. Touch-pain sensation was tested using a neurological brush and pinprick.

After the one-half hour rest period, the area of the median cubital vein of the left arm was swabbed with zephiran chloride, and a fifteen milliliter blood sample was taken from the vein at the left elbow, just prior to the application of ultrasound. A second sample of blood was taken immediately on cessation of the ultrasound application. Third and fourth samples were taken one-half hour and one hour respectively after the application of ultrasound. The blood samples were taken by a laboratory technician.

The ultrasound transducer head was applied to the right forearm of the subject by means of a continuous moving treatment head for the placebo, subthermal and thermal groups. The control group received no treatment but the treatment time was observed so that blood samples might be taken at the appropriate times. The treatment head was moved gently, with slight pressure in a slow circular pattern

FIGURE 4: TESTING AND BLOOD SAMPLING APPARATUS

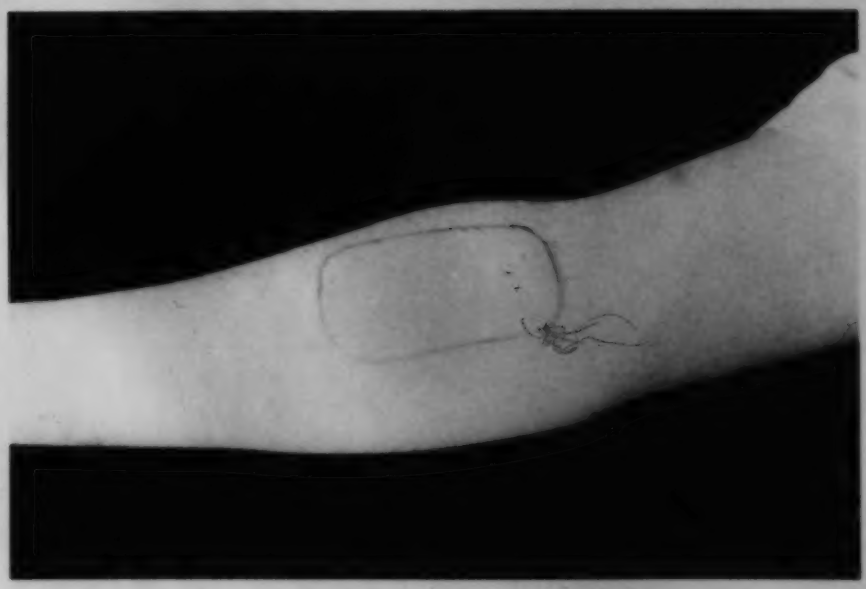

FIGURE 5: AREA OF ARM IRRADIATED WITH ULTRASOUND

FIGURE 6: ULTRASOUND APPARATUS AND TREATMENT COUCH

FIGURE 7: OVERHEAD VIEW OF ULTRASOUND MACHINE

of overlapping circles at a rate of about two inches per second so that there was an even distribution of sound energy (5,6,7,8). The treatment head was kept perpendicular to, and in full contact with the skin to prevent reflection of the sound energy (5,6). Aquasonic gel ® was used as a coupling agent (1). Upon completion of the treatment, the skin was cleaned of all traces of couplant.

On completion of the test, the areas of both arms used in the test were examined for possible injury. Each subject was advised to contact the investigator immediately if they encountered any problems such as infection over the following week.

Before leaving the room, each subject was asked to participate in a subjective sensation test for the two therapeutic dosages of ultrasound using a standing wave field. The treatment head, using aquasonic gel ® as a contact medium, was placed on the dorsal aspect of the right hand. Each subject was given a dosage of 0.5 watts per square centimetre and the time was taken from the time the ultrasound energy was applied to the tissues until the subject subjectively felt pain or tingling. The procedure was repeated using 1.5 watts per square centimetre, and the time recorded.

The blood samples were labelled with the subject's number and the symbol "A-1" for before and "B-1", "B-2" and "B-3" for immediately after, thirty minutes after and one hour after treatment respectively. The data obtained

for each subject was a blood sample showing blood sugar level before application of ultrasound and three blood samples showing blood sugar levels after application of ultrasound at different time intervals. All samples were taken within five minutes of the required time. The blood samples were then taken to the University of Alberta Hospital Laboratory where they were analyzed.

Statistical Treatment

The method used was the two-factor experiment having repeated measures on the same elements as described by Winer. There were several advantages offered by this type of design. It controlled individual differences between experimental units and it provided statistically independent estimates of treatment effects from all cells in the test. In addition, with each subject acting as his/her own control, a smaller sample size could be used.

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CHAPTER IV

Results and Discussion

Results

The purpose of this study was to investigate the effect of therapeutic ultrasound on human blood sugar levels. A sample of forty healthy subjects was studied.

The method used for the statistical analysis of the data was the two factor experiment having repeated measures on the same elements. In the analysis, the terms group and treatment time refer to the following: 1) Group - The subjects were assigned, using a random table, to four groups. The control group received no treatment of ultrasound. The placebo group received an application of ultrasound with the intensity dial reading 0.0 watts per square centimetre. The subthermal group received a treatment of ultrasound with the intensity dial on the ultrasound machine reading 0.5 watts per square centimetre. The thermal group received an administration of ultrasound 1.5 watts per square centimetre as noted on the intensity dial.

2) Treatment time - The time before treatment refers to the blood sample which was taken just prior to the application of ultrasound. The time after treatment refers to the blood sample which was taken within five minutes of the cessation of ultrasound application. The times thirty minutes after

treatment and sixty minutes after treatment refer to the blood samples which were taken thirty minutes and sixty minutes after the administration of ultrasound.

TABLE 1 presents the time treatment means for human blood sugar for each group at different treatment times. Figure 8 shows the table in graphic form.

TABLE 2 shows the values obtained in the analysis of variance using computer program ANOV23 at The University of Alberta. Figure 9 illustrates the mean blood sugar levels for time-treatment interaction. The raw data for the statistical analysis may be found in Appendix C. Figure 10 indicates the mean blood sugar levels for different treatment groups.

Discussion:

From the data in Table 2, there is no significant difference in treatment effects or time effects for the four groups. However, if one looks at the interaction between time and treatment, there is a significant difference at the .05 level. In analysis of the data, the interaction between time and treatment occurs between the control and placebo and between the placebo and the subthermal group. This interaction can be seen in figure 9.

The Technicon AutoAnalyzer One which was used to analyze the blood had a day to day and within day variability of approximately 3.5 per cent. To test the reliability of the machine, the University of Alberta Hospital Laboratory uses

TABLE 1

TIME x TREATMENT MEANS FOR HUMAN BLOOD SUGAR (mg%)

GROUP	BEFORE TREATMENT	AFTER TREATMENT	30 MINUTES AFTER TREATMENT	60 MINUTES AFTER TREATMENT
CONTROL	87.90 σ = 8.92	90.30 σ = 10.53	84.80 σ = 5.74	87.40 σ = 7.63
PLACEBO	89.20 σ = 5.42	87.00 σ = 6.93	89.40 σ = 5.44	89.10 σ = 5.58
SUBTHERMAL	86.10 σ = 4.22	85.20 σ = 4.79	87.20 σ = 7.15	89.80 σ = 4.89
THERMAL	89.60 σ = 5.90	88.50 σ = 7.05	89.40 σ = 5.16	89.30 σ = 6.82

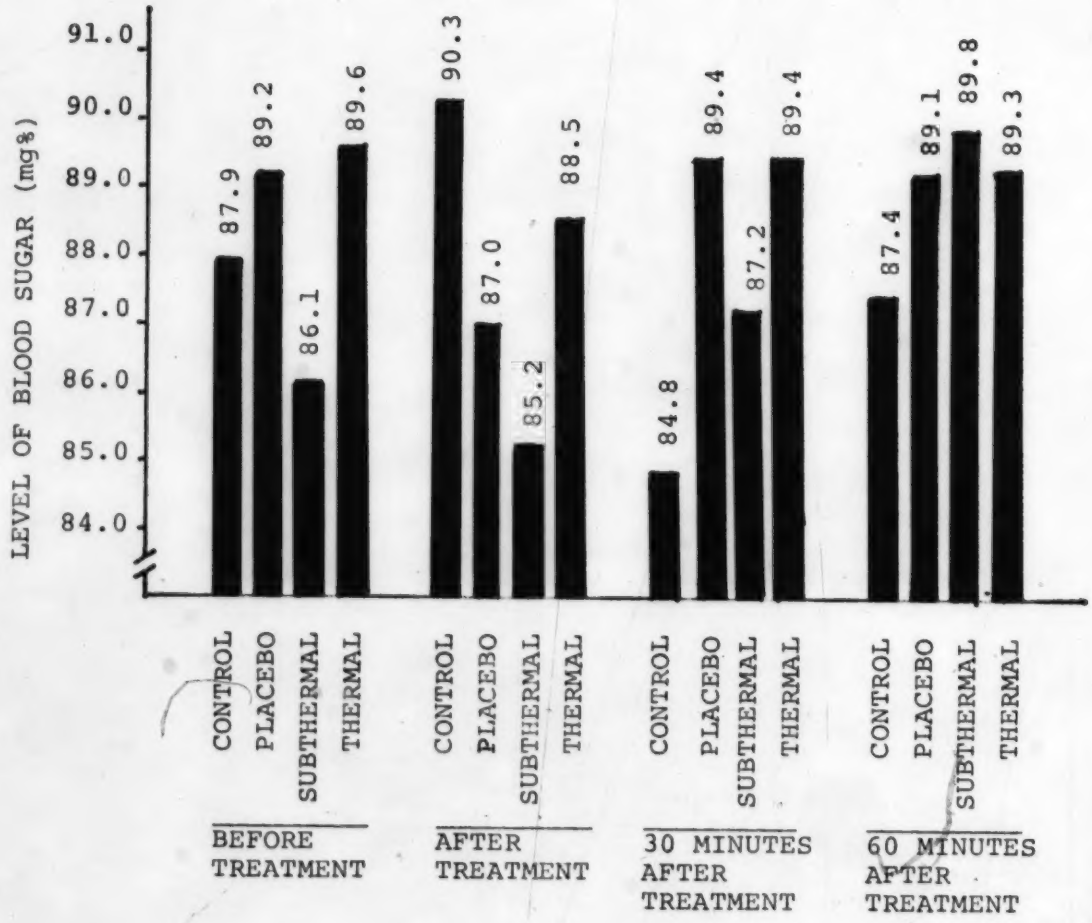

FIGURE 8: MEAN BLOOD SUGAR LEVELS FOR THE FOUR GROUPS AT DIFFERENT TIME INTERVALS

TABLE 2

ANALYSIS OF VARIANCE FOR HUMAN BLOOD SUGAR VALUES

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F. RATIO
BETWEEN SUBJECTS	5722.000	39		
TREATMENT MAIN EFFECTS	113.125	3	37.708	0.242 *
SUBJECTS WITHIN GROUPS	5609.000	36	155.806	
WITHIN SUBJECTS	1651.000	120		
TIME MAIN EFFECTS	38.125	3	12.708	1.027 **
TREATMENT-TIME INTERACTION	278.750	9	30.972	2.504 ***
TIME x SUBJECTS WITHIN GROUPS	1336.00	108	12.370	

* For significant difference at the .05 level, $F \geq 2.86$ (at .01 level: $F \geq 4.38$)
** For significant difference at the .05 level, $F \geq 2.68$ (at .01 level: $F \geq 3.95$)
*** For significant difference at the .05 level, $F \geq 1.96$ (at .01 level: $F \geq 2.56$)

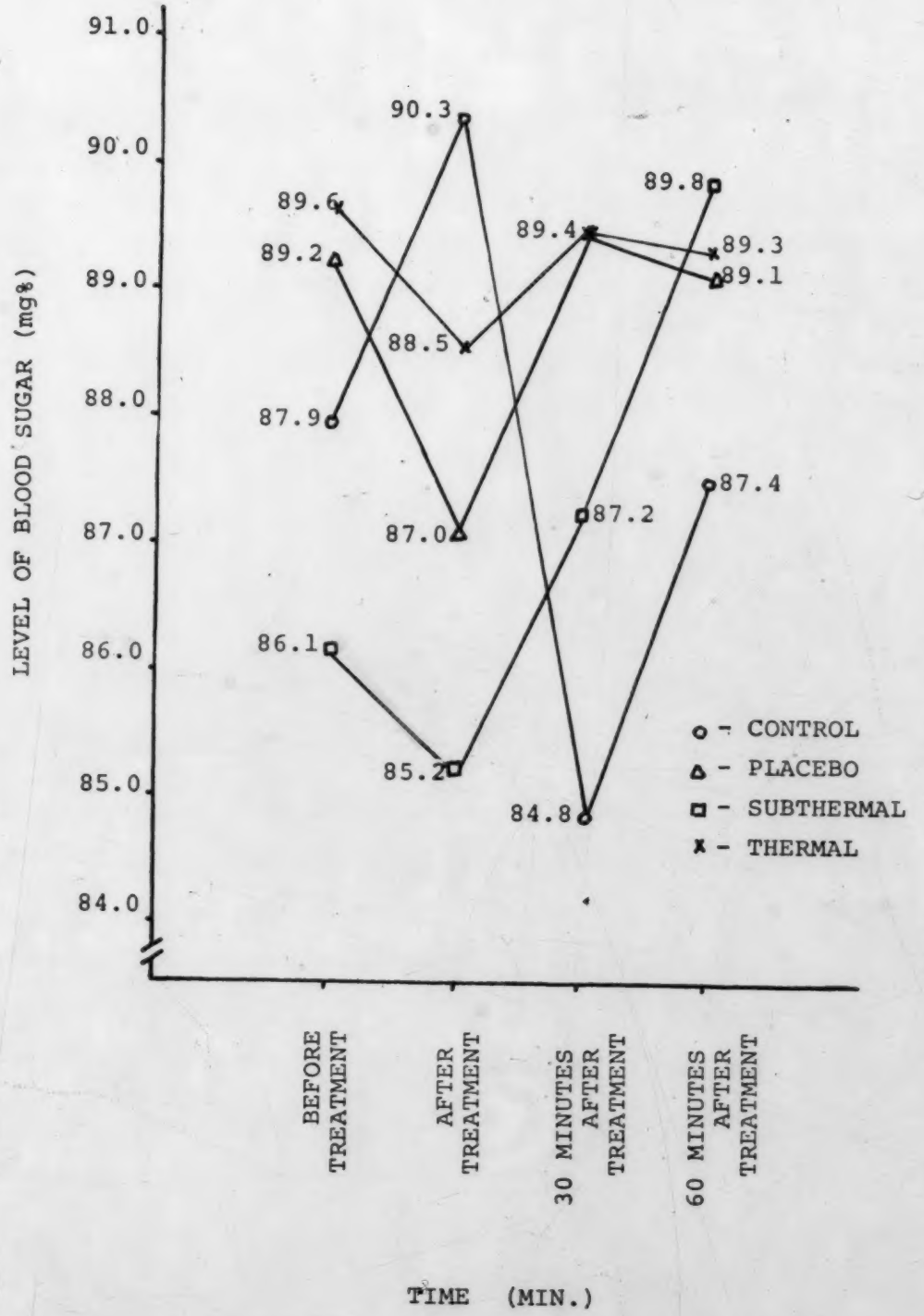

FIGURE 9: MEAN BLOOD SUGAR LEVELS FOR TIME-TREATMENT INTERACTION

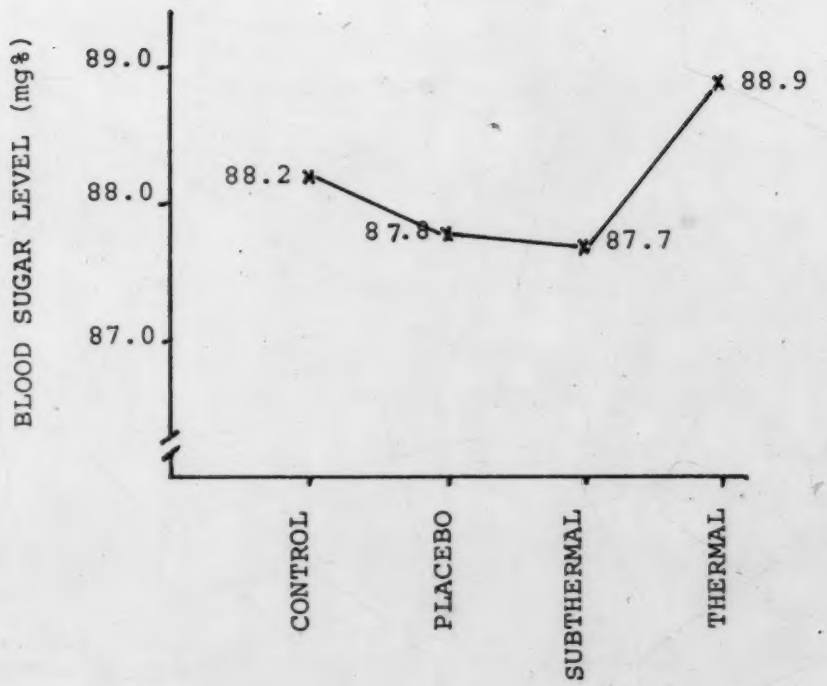

FIGURE 10: MEAN BLOOD SUGAR LEVELS FOR CONTROL AND TREATMENT GROUPS

a known sample of 188 milligram per cent sugar and allows a value of plus or minus six milligram per cent at two standard deviations.

In the test, the control group showed a variability of 5.5 milligram per cent which is less than the variability allowed by the AutoAnalyzer. Also, the control group, which received the same treatment as the other groups except for the application of ultrasound, showed greater variability than the placebo or the two treatment groups.

The interaction between the control group and the placebo group was significant at the 0.5 level of significance. No plausible explanation can be put forth for this result and it is hypothesized that this significant difference is a chance statistical aberration.

The action of the application of ultrasound did not have a significant effect, overall, on the level of blood sugar. The results obtained in this study differ greatly from those cited by Summer and Patrick (1). They state that the drop in blood sugar was obvious after the treatment of ultrasound. They state that the average drop in blood sugar was 15.7 milligram per cent and a maximum drop in blood sugar of fifty-seven milligram per cent is noted. Furthermore, they state that the decrease in the level of blood sugar was as much as from 101 to eighty-two milligram per cent. In stating these figures, Summer and Patrick do not give the dosage of ultrasound used, the area of application, the treatment time, or the size or type of the subjects.

Two authors (2,3) state that ultrasound changes the composition of carbohydrates. However, the doses of ultrasound used to cause this change are well above the therapeutic range and the time of insonation is much greater than normal treatment time.

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CHAPTER V

Summary and Conclusions

Purpose

The purpose of the study was to determine what effect, if any, a subthermal and thermal dosage of therapeutic ultrasound would have on the level of blood sugar in the human body.

Hypothesis

The following null hypothesis was assumed throughout the study:

If continuous ultrasound energy with a frequency of 870 kilohertz is applied as a subthermal and thermal dosage with continuous output to the right forearm of normal healthy subjects for a period of ten minutes using aquasonic gel® as a coupling agent, there will be no significant change or effect on the level of blood sugar in the human body at the .05 level of significance immediately after, thirty minutes after, or one hour after treatment - symbolically

$$\mu_1 = \mu_2 = \mu_3 = \mu_4$$

Procedures

The pre-test procedures involved the following steps:

1] Subjects were asked not to eat, smoke, or drink anything but water eight hours prior to the test.

2) Subjects were asked to lie on the bed for thirty minutes prior to treatment. During this time, they were asked to fill out a medical questionaire, read three sheets on information to potential subjects, and to sign an informed consent form. During the time period, they also had their hot-cold, and touch-pain sensation tested and the outline of the template was drawn on the right forearm.

3) Immediately prior to the treatment, a blood sample was taken.

4) Each subject received a ten minute treatment of a predetermined dosage of ultrasound while lying down. The control group received no application of ultrasound but was timed for proper sequence of taking of blood samples. The placebo group received an administration of ultrasound with the intensity of ultrasound turned off. The subthermal group received a treatment of ultrasound with the intensity dial on the ultrasound machine reading 0.5 watts per square centimetre. The thermal group received an application of ultrasound of 1.5 watts per square centimetre as noted on the intensity dial.

5) Blood samples were then taken immediately after the treatment time, and thirty minutes, and sixty minutes after the treatment while the subject was lying down.

Results

The results indicate the application of therapeutic ultrasound does not significantly effect the level of blood sugar in the human body. This result appears to hold true for therapeutic subthermal and therapeutic thermal doses of ultrasound which are given for ten minutes in an area four times the size of the ultrasound treatment head to healthy human beings.

Specifically, the results indicate that the null hypothesis is accepted. Any changes in the level of blood sugar which did occur fall within the range of normal variability. That is, any change in blood sugar noted in the treatment groups was within the range of variability of the control group.

Conclusions

Using an ultrasound apparatus of 870 kilocycles per second and taking blood samples immediately before the treatment, immediately after the treatment, thirty minutes after the treatment, and sixty minutes after the treatment, the following conclusions were made:

1. There is no significant difference in the level of blood sugar in the human body upon the application of therapeutic ultrasound using dosages of 0.5 and 1.5 watts per square centimetre.
2. It is hypothesized that the significant difference in the interaction between the control and placebo was due to chance.

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Appendix A

Forms Used in Research Project

ALLOCATION OF SUBJECTS TO GROUPS

SUBJECT NUMBER	NAME	GROUP

TO: Volunteers who have agreed to participate in the study to examine the effects of application of therapeutic ultrasound on the level of blood sugar in the human body.

Dear

Thank you for being willing to act as a subject in this study. It has been possible to arrange your test appointment at one of the times suggested by you.

Your appointment will be on

_____ at _____.

I would be grateful if you would let me know in advance if, for any reason you are unable to attend for this appointment. You may get in touch with me either by leaving a message in my mail box at Corbett Hall or by telephoning my office -- 432-5985.

You are reminded that you should not eat, drink or smoke for a period of eight hours prior to the start of your test session.

Please wear P.E. kit for the test session.

I look forward to seeing you.

Yours sincerely,

David Magee

TO: Each person who has agreed to participate in the study to examine the effects of application of therapeutic ultrasound on the level of blood sugar in the body.

FROM: David Magee

Thank you for being willing to participate in this study.

In order that the results of the study shall be as valid as possible, I would be grateful if you would complete the details below. I assure you that these personal details will not be disclosed at any time to any third person in such a way that you could be identified from them.

PERSONAL DATA FORM

Name:

Address:

Phone number:

Age: _____years _____months

Weight: _____pounds

Height: _____inches

SENSATION TESTS (to be filled in by investigator):

1. Hot-cold sensation:

2. Touch-pain sensation:

Please indicate by answering YES or NO in the appropriate column whether you or any member of your immediate family have, to the best of your knowledge, ever suffered from any of the following medical problems: -

	SELF	FAMILY
Heart Disease		
Raynauds Phenomenon		
Thrombosis		
Varicose Veins		
Swollen Ankles		
Pins and Needles in Hands or Feet		
Eczema		
Asthma		
Hay Fever		
Allergic Reactions to Drugs		
Diabetes		
Neuritis		

Do you smoke cigarettes? ____. If so, approximately how many per day?___.
Have you given blood in the last year? _____
If so, when? _____
Are you presently taking medication for any reason? _____.

Date: _____ Signed: _____

Information to Potential Subjects

The testing session for each subject will take approximately one and a half hours at a single attendance. No pre-training is required for participation in this study which is concerned with the effect of therapeutic ultrasound on the level of blood sugar in the human body.

If you agree to participate in this study you will be asked not to eat, smoke, or drink for a period of eight hours prior to your arrival in the testing laboratory.

One group of subjects will act as a control group and will receive no application of ultrasound. For all other subjects, the following treatment applies.

Zephiran Chloride will be used to clean the area to be treated and the area from which the blood sample is taken.

A small amount of couplant jelly will be applied to your forearm area. The treatment head of the ultrasound apparatus will be kept in contact with the skin while being moved slowly over the area for a period of ten minutes. During this time you will receive a pre-determined dose of ultrasonic energy. At the end of the application the treatment head will be removed and the skin cleaned of all traces of the couplant material.

While the apparatus is switched on you may feel nothing, or a sensation of mild warmth immediately beneath the treatment head. You should not experience discomfort at any time during or following the test period. The doses of ultrasonic energy which will be used in this experiment are well within

the intensity range in which treatments have been given over many years to patients attending for physical therapy without producing either discomfort or injury. Very occasionally, however, a person receiving ultrasound may complain of parasthesia or pain during the treatment either immediately beneath the treatment head or in some other area of the body. You should inform the investigator if you experience any other sensation but that of mild warmth.

Prior to the application of ultrasound a small sample of blood will be taken using a hypodermic needle and tourniquet by a competent laboratory technician. As well, small samples of blood will be taken immediately after the application of ultrasound, one half hour after application and one hour after application. Each sample will consist of fifteen (15) ml. of blood. At the end of this time, you will be free to leave. Control subjects will have blood samples taken at the same interval.

It often happens that following ultrasound treatment some feeling of heaviness in a limb or a general tiredness may be felt. This is purely temporary. Although you do not need to worry about either of these sensations the investigator should be informed of their occurence.

The purpose of this information is to ensure that potential subjects are informed of, and fully understand the procedures and potential hazards to which they will be exposed if they agree to participate in the study. If you have questions please discuss these with the investigator before

you sign the Informed Consent Form.

Should you so choose, you may end your participation in this study at any time without being required to explain your reasons for withdrawal.

All personal information given to the investigator during this study will be regarded as confidential. Your anonymity will be protected by the use of a coding system for subject identification. Only the investigator will have the key to this code.

INFORMED CONSENT FORM FOR RESEARCH STUDY

I, _____, hereby give my consent to participate in research study on the effect of therapeutic ultrasound on the level of blood sugar in the human body, the general plan of which has been explained to me including anticipated benefits, risks, and potential complications.

I fully understand as it has been explained to me that by notice given to the undersigned principal investigator that I may withdraw from this research project anytime that I may elect to do so.

_____
Participant's Signature

I hereby certify that I have given to the above individual an explanation of the contemplated study and its risks and potential complications.

_____
Principal Investigator

I, _____, certify that I was present at the time the above explanation was given in English and in my opinion the subject understood the factors involved. I also witnessed the signatures of both parties above.

_____ WITNESS _____
Date

Daily Blood Sample Timetable

Time	Group	Subject Name	Subject Number	Sample Number

Daily Ultrasound Timetable

Time	Group	Subject Name	Subject Number

Table for Timing of Subjective Feeling of Ultrasound

Subject Number	Group	Time for 0.5w/cm^2 (sec.)	Time for 1.5w/cm^2 (sec.

FORM ACCOMPANYING BLOOD SAMPLE TO UNIVERSITY OF ALBERTA HOSPITAL LABORATORY

PROJECT: Effect of Therapeutic Ultrasound on Human Blood Sugar Levels

PROJECT NO: DJM-1-75-U.S.B.S.

PROJECT INVESTIGATOR: David J. Magee
School of Rehabilitation Medicine
University of Alberta
Edmonton, Alberta
Phone: 432-5985

SUBJECT NUMBER: . . . . . .

SAMPLE READINGS: A-1:. . . . . .

B-1:. . . . . .

B-2:. . . . . .

B-3:. . . . . .

Laboratory Worksheet

Group Intensity: _____

Subject Number	Blood Sugar Level			
	Before Test	Immediately After Test	One Half Hour After Test	One Hour After Test

Appendix B

Description of Blood Sugar Analysis Method

Simultaneous Glucose-Urea Nitrogen Analysis

Introduction:

In this procedure a double manifold is constructed, combining the two single manifolds as proposed by Technicon for individual determinations of glucose and urea nitrogen. The method of double dialysis allows the use of only one sample and yet maintains the desired degree of sensitivity in both determinations. In this study, one is interested only in the Glucose Measurement.

Glucose is determined by the potassium ferricyanide-potassium ferrocyanide oxidation reduction reaction. The yellow potassium ferricyanide solution is reduced to colorless ferrocyanide. The reduction in color is proportional to the amount of glucose contained in the specimen and is measured at 420 mu in a colorimeter equipped with a flow cuvette which has a 15 mm. light path.

Urea nitrogen is determined by a modification of the carbamide-diacetyl reaction. It is based on the direct reaction of urea and diacetyl monoxime under acidic conditions. Diacetyl monoxime is hydrolyzed to diacetyl which reacts directly with urea in the presence of the acid reagent to form Triazine derivatives by an oxidative condensation reaction. The presence of thiosemicarbazide intensifies the color of the reaction product, eliminating the need of concentrated acid reagents. The colored product of the reaction is measured at 530 mu in a colorimeter with a 15 mm. light path flow cuvette.

Reagents

1. Saline (used in Glucose Determination):

Sodium chloride	9.0 gm
Distilled H_2O q.s.	1000 mls

Place the NaCl in a one litre volumetric flask. Disolve in about 500 mls of distilled water, dilute to volume with water. Mix. Transfer to 1 litre polyethylene bottle, add 0.5 ml Brij-35.

2. Alkaline Potassium Ferricyanide (used in Glucose Determination):

Sodium chloride	9.0 gm
Potassium Ferricyanide	0.25 gm
Sodium Carbonate	20.0 gm
Distilled Water	1000 ml

Place the NaCl in a 1 litre volumetric flask, dissolve in approximately 500 mls distilled water. Carefully weigh out the potassium ferricyanide and add to the NaCl solution. Mix until dissolved. Add 20 gm. Sodium Carbonate to the above mixture and stir with a magnetic stirrer until solution is complete. Bring to 1000 ml with distilled water. Mix well and transfer to an amber polyethylene bottle, add 0.5 ml Brij-35.

3. Stock Diacetyl Monoxime (used in Urea Nitrogen Determination):

Diacetyl Monoxime (2,3-Butanidione-2-oxime)	25 gm.
Distilled water q.s.	1000 ml.

Place diacetyl monoxime in a one litre volumetric flask. Add approximately 600 ml distilled water and shake until the diacetyl monoxime is completely dissolved. Dilute to volume with distilled water. Mix and filter. Store in amber bottle.

4. Stock Thiosemicarbazide (used in Urea Nitrogen Determination):

Thiosemicarbazide (T.S.C.)	5 gm.
Distilled water q.s.	1000 ml.

Place thiosemicarbazide in a one litre volumetric flask. Add approximately 600 ml distilled water and mix until T.S.C. is completely dissolved. Dilute to mark with distilled water. Store in amber bottle.

5. Working BUN Color Reagent (used in Urea Nitrogen Determination):

Stock Diacetyl Monoxime	67 ml.
Stock Thiosemicarbazide	67 ml.
Distilled water q.s.	1000 ml.

Add Stock Diacetyl Monoxime and Stock Thiosemicarbazide to approximately 300 ml. distilled water in a one liter volumetric flask. Dilute to volume. Add 0.5 ml. Brij-35. Mix. Store in an amber bottle.

6. Stock Ferric Chloride - Phosphoric Acid. (used in Urea Nitrogen Determination):

Ferric Chloride ($FeCl_3.6H_20$)	15 gm.
Phosphoric Acid 85%	300 ml.
Distilled water q.s.	450 ml.

Dissolve ferric chloride in 30 ml. distilled water in a 500 ml graduated mixing cylinder. Add phosphoric acid slowly; while mixing q.s. to 450 ml. with distilled water. Mix. Store in amber bottle.

7. Stock Sulfuric Acid 20% (used in Glucose Determination)

Conc. sulfuric acid	200 ml.
Distilled water q.s.	1000 ml.

While mixing, add sulfuric acid to approximately 600 ml distilled water in a 1 litre volumetric flask. Allow to cool. Dilute to mark with distilled water. Mix.

8. Working BUN Acid (used in Urea Nitrogen Determination)
   Stock Ferric Chloride-Phosphoric Acid 1 ml.
   Stock Sulfuric Acid 20% 1000 ml.
   Place approximately 500 ml. stock 20% sulfuric acid in a one litre volumetric flask. Add 1 ml. of the stock ferric chloride-phosphoric acid, mix. Dilute to volume with stock 20% sulfuric acid. Mix.

STANDARDS

1. Stock Urea Nitrogen.
   Standard diluent: Phenylmercury acetate PMA 0.20 gm.
   Sulfuric acid (cmc) 1.4 ml
   Distilled water q.s. 5000 ml
   Add 100 ml. of distilled water to 0.2 gm. PMA in a 250 ml. beaker. Heat until PMA dissolves. After cooling transfer quantitatively to a 5 litre volumetric flask. Add 1.4 ml concentrated H_2SO_4, mix. Dilute to volume with distilled water.

   For Stock Urea Nitrogen Standard (10 mg/1ml):
   Urea C.P., A.C.5. 21.433 gm.
   Standard diluent q.s. 1000 ml.
   Carefully weigh out urea and place in a 1 litre volumetric flask. Add approximately 500 ml. standard diluent and mix until dissolved. Q.s. to volume with standard diluent. Mix well. Store in amber polyethylene bottle in refrigerator.

2. Stock Glucose (10 mg/ml)
   Dextrose (Anhydrous) C.P., A.C.S. 20.0 gm.
   Saturated Benzoic Acid q.s. 2000 ml.
   Carefully weigh out 20.0 gm. dextrose and transfer to a 2-litre volumetric flask. Add some saturated benzoic acid and mix until dissolved. Q.s. to volume with saturated benzoic acid. Mix well. Store in amber bottle in refrigerator.

3. To prepare working standards:

Standard	Stock glucose 10 mg/1 ml	Stock urea 10 mg/ml	PMA diluent q.s.	Final conc. glucose-urea
S_1	5 ml	2 ml	200 ml	25 - 10 mg%
S_2	10 ml	2 ml	200 ml	50 - 10 mg%
S_3	20 ml	6 ml	200 ml	100 - 30 mg%
S_4	30 ml	10 ml	200 ml	150 - 50 mg%
S_5	40 ml	14 ml	200 ml	200 - 70 mg%
S_6	50 ml	18 ml	200 ml	250 - 90 mg%
S_7	60 ml	24 ml	200 ml	300 - 120 mg%

Store all working standards in refrigerator when not in use.

OPERATING PROCEDURE

1. Turn on both colorimeters and recorders. Allow to warm up for 15 minutes before starting the run. Have all manifold lines pumping water during this warm-up period.

2. With 420 mu filters in the glucose colorimeter and reagent lines drawing H_2O, turn on chart drive on glucose recorder. Using the 100%T knob on the colorimeter adjust the base line to 99%T (use any slit which gives a reading on the dial between 200-800). Place a zero slit in front of the sample filter in the colorimeter and check the O-reading on the graph, if necessary adjust pen to zero %T with the O adjustment knob on colorimeter. Remove O-slit.

3. Place all lines in reagents, allow sample line to draw water.

4. When reagents reach the glucose colorimeter the base line should fall to 13 $\leq$ 3%T. If not adjust the ferricyanide agent - see note # (i).

5. Turn on chart drive on BUN recorder. Set the pen at 99%T using the 100%T dial on the colorimeter and any slit which gives a reading between 200-800 on this dial. Use 530 mu filters in the colorimeter.

   Check the 0%T reading on the graph by placing an O-slit in front of the sample filter and make any necessary adjustment with the O-adjustment knob on the colorimeter. Remove the O-slit, the pen should return to 99%T, if not, adjust it to 99%T using the 100%T dial.

6. Set up the sample plate with a series of standards first S_1-S_7. The S_3 is run immediately following the series of standards, after the controls, and every tenth cup thereafter throughout the run. It should not vary more than $\leq$ 3 mg% for BUN and $\leq$ 8 mg% for glucose.

7. Controls are run at the beginning, in the middle and at the end of the run. Both the normal Lab Pool and the Hi Glucose Pool are run for controls.

8. Begin to aspirate samples at the rate of 60 specimens per hour.

CALCULATIONS

After completion of the run standard graphs are drawn on general purpose comparator and the corresponding values for glucose and BUN in the unknowns are read off these graphs and recorded.

NORMAL RANGE and S. D.

BUN - normal range = 7 - 18 mg%
2 S.D. = $\leq$ 3 mg%

GLUCOSE - normal range (fasting) = 65 - 105 mg%
2 S.D. = $\leq$ 8mg%

NOTES

(i) If the glucose reagent baseline is higher than 16% a stock solution of 5% potassium ferricyanide in 0.9% NaCl is added. If the baseline is lower than 10%T dilute the ferricyanide reagent with 2% sodium carbonate in 0.9% NaCl.

(ii) Blood specimens are drawn in vacutainers containing sodium fluoride as an anti-coagulant and glucose preservative. These specimens are spun down and the plasma, free of cells, is used for the determinations. NB. Do not use whole blood.

(iii) Samples are labeled as to time of collection and if the patient is fasting. This information must be transferred to the work book and requisition in order to allow interpretation of the glucose result.

Appendix C

Raw Data

GROUP: Control

Subject Number	Blood Sugar Level (mg%)			
	Before Test	Immediately After Test	One Half Hour After Test	One Hour After Test
004	76	76	81	79
023	82	89	82	82
029	80	78	81	80
032	84	89	77	80
035	81	80	83	84
101	107	113	98	103
102	91	98	83	92
103	98	98	91	90
104	88	88	84	87
105	92	94	88	97

GROUP: Placebo

Subject Number	Blood Sugar Level (mg%)			
	Before Test	Immediately After Test	One Half Hour After Test	One Hour After Test
006	85	82	80	88
007	95	92	95	92
009	89	82	85	85
010	92	98	91	92
022	90	90	89	90
106	90	88	88	81
107	97	97	97	97
108	92	83	95	89
110	85	83	92	97
124	77	75	82	80

GROUP: Treatment Group Receiving 0.5w/cm^2 of ultrasound

Subject Number	Blood Sugar Level (mg%)			
	Before Test	Immediately After Test	One Half Hour After Test	One Hour After Test
012	81	78	79	86
013	88	86	85	94
021	88	86	91	91
024	84	80	77	83
034	91	89	88	90
111	84	87	85	98
113	92	88	97	94
114	91	95	101	93
122	81	82	82	83
127	81	81	87	86

GROUP: Treatment Group Receiving 1.5w/cm^2 of ultrasound

Subject Number	Blood Sugar Level (mg%)			
	Before Test	Immediately After Test	One Half Hour After Test	One Hour After Test
017	85	87	84	87
020	88	84	95	93
031	83	81	87	78
040	83	82	85	82
041	88	83	90	95
116	97	101	96	102
119	100	97	97	94
121	85	83	81	84
123	97	89	92	92
125	90	98	87	86

Appendix D

Timing for Subjective Feeling of Ultrasound

As there was no instrument available to test the output of the ultrasound apparatus, the two treatment doses were timed for each dosage of ultrasound - 0.5 and 1.5 watts per square centimetre. The first dosage, 0.5 watts per square centimetre is considered a subthermal dose while the second dose, 1.5 watts per square centimetre. Analysis showed there was a significant difference in time for subjective feeling of ultrasound between the two doses.

Timing for Subjective Feeling of Ultrasound

Subject Number	Group	Time (Sec) For 0.5w/cm^2 Dosage	Time (Sec) For 1.5w/cm^2 Dosage	Subject Number	Group	Time (Sec) For 0.5w/cm^2 Dosage	Time (Sec) For 1.5w/cm^2 Dosage
004	1	5	1	012	3	7	0.5
023	1	10	1	013	3	5	1
029	1	3.5	1	021	3	1.5	1
032	1	3	0.5	024	3	3	0.5
035	1	60	1.5	034	3	12.5	1
101	1	14	1	111	3	7.5	2
102	1	20	1.5	113	3	9.5	1
103	1	37	1	114	3	4	1.5
104	1	5	1	122	3	4	1.5
105	1	2.5	0.5	127	3	35	1
006	2	0.75	0.5	017	4	5	1
007	2	26.5	2	040	4	2	1.5
009	2	38	2	041	4	7	2
010	2	5	1.5	020	4	28	1.5
022	2	31	4	031	4	1.25	1
106	2	20	3	116	4	1.5	0.5
107	2	2	1	119	4	7.5	0.5
108	2	4.5	1	121	4	9.5	1
110	2	2	0.75	123	4	2	1
124	2	2	0.5	125	4	20	1

To determine if there is a significant difference between the two doses of ultrasound one may use the Significance of the Difference between two means for correlated samples as described by George A. Ferguson* in "Statistical Analysis in Psychology and Education".

$$t = \frac{\Sigma D}{\sqrt{\frac{[N\Sigma D^2 - (\Sigma D)^2]}{N - 1}}}$$

$$= 5.03$$

* In a two tailed test, one needs $t \geq 2.021$ for a significant difference between the two means. Thus there is a significant difference between the two doses of ultrasound.

* McGraw-Hill Book Company, New York, 1971.